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^{41}Ca and Accelerator Mass Spectrometry to Monitor Calcium Metabolism in End Stage Renal Disease Patients

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Background: **Monitoring bone resorption with measurements of bone density and biochemical markers is indirect. We hypothesized that bone resorption can be studied directly by serial measurements of the ratio ^{41}Ca/Ca in serum after in vivo labeling of calcium pools with ^{41}Ca. We report the preparation of an intravenous ^{41}Ca dose suitable for humans, an analytical method for determining ^{41}Ca/Ca isotope ratios in biological samples, and studies in human volunteers.**

Methods: **^{41}Ca was formulated and aliquoted into individual vials, and to the extent possible, the ^{41}Ca doses were tested according to US Pharmacopeia (USP) guidelines. A 10 nCi dose of ^{41}Ca was administered intravenously to 4 end stage renal disease (ESRD) patients on hemodialysis and 4 healthy control individuals. Distribution kinetics were determined over 168 days. Calcium was isolated with 3 precipitation steps and a cation-exchange column, and ^{41}Ca/Ca ratios in serum were then measured by accelerator mass spectrometry.**

Results: **The dosing solution was chemically and radiologically pure, contained <0.1 endotoxin unit/mL, and passed USP sterility tests. Quantification of ^{41}Ca/Ca ratios was linear from 6×10^{-14} to 9.1×10^{-10}. The run-to-run imprecision (as CV) of the method was 4% at 4.6×10^{-11} and 6% at 9.1×10^{-10}. The area under the curve of ^{41}Ca in the central compartment vs time was significantly less for ESRD patients than for controls (P <0.005).**

Conclusions: **Isotope ratios spanning 5 orders of magnitude can be measured by accelerator mass spectrometry with excellent precision in the range observed in samples collected from patients who have received 10 nCi of ^{41}Ca. The ^{41}Ca at this dose caused no adverse effects in 8 volunteers. This is the first report of the use of ^{41}Ca to monitor differences in bone turnover between healthy individuals and ESRD patients.**

Calcium exists naturally as 6 stable isotopes, with the most abundant isotope being ^{40}Ca. Stable (^{42}Ca, ^{44}Ca, and ^{46}Ca) and short-lived radioactive isotopes (^{45}Ca and ^{47}Ca) of calcium have been used to study calcium metabolism for years *(1–3)*. Limitations of the stable isotopes are that they are expensive to purify and that relatively large doses need to be given to distinguish the exogenously administered tracer from the naturally occurring signal. Limitations of the short-lived radioisotopes are that they deliver significant amounts of radiation and that their short half-lives prevent long-term tracing. The long-lived radioisotope, ^{41}Ca, is an ideal candidate for biological tracer studies because the background signal of ^{41}Ca is essentially zero as a result of the very low natural production of ^{41}Ca. The advantage of combining a marker such as ^{41}Ca (low noise) with a technique with attomole sensitivity, such as accelerator mass spectrometry (AMS),[6] is that excellent signal/noise ratios can be obtained from nanogram quantities of isotopes administered *(4–12)*.

^{41}Ca has a half-life of 104 000 years and decays by

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[6] Nonstandard abbreviations: AMS, accelerator mass spectrometry; ESRD, end stage renal disease; AP, alkaline phosphatase; USP, United States Pharmacopeia; and PTH, parathyroid hormone.

electron capture to the naturally occurring and stable isotope ^{41}K, emitting x-rays and electrons of very low energy (<3.6 keV) in the process *(13, 14)*. For biological experiments, ^{41}Ca is typically prepared by irradiating calcium samples with neutrons in a nuclear reactor. Other radioisotopes of calcium, ^{45}Ca and ^{47}Ca, are also formed during this process, but these have relatively short half-lives (163 and 4.5 days, respectively). After sufficient time is allowed for the ^{45}Ca to decay, the only radioactive material remaining is ^{41}Ca. The long lifetime of ^{41}Ca prevents its routine analysis by decay counting, but AMS is particularly well suited for long-term monitoring of bone resorption by direct quantification of ^{41}Ca concentrations. The long half-life of ^{41}Ca, combined with the sensitivity of AMS, allows isotope ratios to be measured for a period of years, a time span that is not feasible with other isotopes of calcium. Potential clinical uses of ^{41}Ca include monitoring of bone loss in patients with end stage renal disease (ESRD), diagnosis of osteoporosis, monitoring of the effectiveness of antiresorptive drugs, and early detection of bone metastasis.

Biochemical markers of bone disease have been in use for more than 30 years, but although beneficial in certain settings, all have drawbacks, primarily related to analytical and biological variability *(15–19)*. One method for interpreting bone markers involves looking for the "least significant change", which incorporates both the analytical and biological variability. The least significant change amounts for bone alkaline phosphatase (AP), osteocalcin, pyridinoline, deoxypyridinoline, and N-telopeptide are in the range of 15% to 40% *(20)*. Bone mineral density, as measured by dual energy x-ray absorptiometry, provides a quantitative measure of the mineralization of bone but is relatively insensitive to change, generally taking 12 to 18 months before changes can be measured. A widely used diagnostic method for identifying bone disease is biopsy and histomorphometry; however, this procedure has morbidity and, in the absence of severe bone disease, is not clinically indicated *(21)*. We report a novel technique for directly assessing the long-term turnover of the mineral phase of bone by measuring $^{41}Ca/Ca$ ratios in serum after an intravenous dose of 10 nCi of ^{41}Ca.

Materials and Methods

In keeping with general standards used in isotope abundance work, we report $^{41}Ca/Ca$, where Ca reflects all isotopes of calcium.

PREPARATION OF THE DOSING MATERIAL

Initial purification and isotopic analysis of the ^{41}Ca material used for the dosing solution have been described *(22)*. We used an aliquot of the ^{41}Ca master stock solution (described as "C1" in the original publication) as the starting material for the dosing solution *(22)*. Radiologic purity is virtually guaranteed from the lack of significant elemental contaminants and the long decay period (the original dose material was purchased in 1984). However, to ensure that we had no radioactive contaminants, we measured the emitted radiation with a gamma/beta survey meter and a high-purity germanium detector.

We received 2.060 g of a solution of $CaCO_3$ in 4 mol/L HNO_3 containing 0.7034 mg of calcium per gram of solution. By weight, the ^{41}Ca abundance in the solution was 1.232% *(22)*; consequently, this solution contained 18 μg of ^{41}Ca or 1500 nCi. The target for the dosing solution was 10 nCi of ^{41}Ca in each vial as a 9-mL solution. In a sterile hood, using sterile technique, we added ~500 mL of 0.9% sodium chloride injection (9 g/L) USP (Baxter Healthcare) and 3.75 mL of 10% USP $CaCl_2$ (0.9 mol/L; American Reagent Labs) to a 2-L sterile vessel. The 2.060 g of ^{41}Ca solution was quantitatively transferred to this vessel, and the pH was adjusted to 5–7 by addition of 7.5% sodium bicarbonate USP (0.9 mol/L; American Reagent Labs). The remainder of the solution was diluted to 1350 mL by weight. In the sterile hood, the dosing solution was filtered through a 0.2 μm filter (Corning). With the solution still in the sterile hood, we volumetrically pipetted 9-mL portions into 10-mL clear vials (Wheaton Scientific). A gray straight plug (Wheaton Scientific) and aluminum seal (Wheaton Scientific) were placed loosely on the vial and held in place with a piece of autoclave indicating paper. The doses were autoclaved at 121 °C and 20 psi (140 kPa) for 30 min. In addition to the autoclave indicating tape, the autoclave process was monitored with a biological indicator (3M Attest) to determine that sterilization was successful. Immediately after autoclaving, the aluminum seals were crimped in the sterile hood. Representative samples were analyzed for endotoxins and sterility as described below. Throughout the process, plastic containers and pipetting devices were used to decrease the chance that trace metals would be introduced into the dosing solution. Pyrogenicity (endotoxin testing), sterility, bacterial, and fungistasis testing were then performed according to United States Pharmacopeia (USP) guidelines *(23, 24)*. Elemental composition (purity) was checked by inductively coupled plasma/mass spectrometry using good manufacturing procedures (GMP). Inductively coupled plasma/mass spectrometry was chosen for purity testing because this method has excellent sensitivity (<1 μg/L for most elements), is broad spectrum (able to detect over 60 elements), and is the type of analysis best suited to identify potential impurities in the ^{41}Ca dosing solution. There are no USP guidelines for acceptable limits of impurities for ^{41}Ca dosing solutions; we therefore chose a conservative limit of <0.00001% for any heavy metal contaminant [i.e., the maximum amount of heavy metals to which individuals would be exposed would be 0.00009 g (90 μg)].

Radiologic purity of the ^{41}Ca master stock solution (1.5 μCi) was determined with a gamma/beta survey meter and with a high-purity germanium detector. To obtain an energy spectrum of the electron capture decay of ^{41}Ca to ^{41}K, the entire 1.5 μCi of ^{41}Ca was placed in a high-purity germanium detector for 1 week.

PATIENTS

The human subject protocol was approved by Institutional Review Boards at the University of California's Human Research Protections Program and Lawrence Livermore National Laboratory. ESRD patients were selected as the study group because 75%–100% of these patients have bone disease *(25)*, and this study was designed as a proof-of-principle investigation. Our aim was to determine whether $^{41}Ca/Ca$ ratios changed more rapidly in patients with known bone disease than in controls without known bone disease. To further enhance the differences between our control and study groups, we chose patients with high-turnover bone disease as evidenced by increased concentrations of parathyroid hormone (PTH). Because the absorption of ingested calcium in ESRD patients is known to vary widely, we chose to administer the dose intravenously to ensure that all controls and patients received the same initial dose of ^{41}Ca.

Eight human volunteers participated in the study, 4 controls and 4 ESRD patients. All volunteers gave written informed consent. Vital signs and pain rating (0–10) of the volunteers were obtained before administration of 10 nCi of ^{41}Ca via a heparin lock. To further ensure sterility, the entire 9 mL of solution to be administered was aspirated through a 0.22 μm pore-size sterile filter into a 10-mL syringe. The sterile filter was removed, and the dose was administered by slow intravenous push through a heparin lock. The heparin lock was then flushed with 10 mL of normal saline. The heparin lock was removed, and vital signs and pain scores were obtained after administration. The ESRD patients received hemodialysis 3 times weekly with 3.5–4 h per treatment. Their dialysis was performed with high-flux membranes, and their calcium management followed K-DOQI guidelines *(26)*. ^{41}Ca was administered immediately after dialysis treatment.

SAMPLE ANALYSIS

Samples were analyzed at the Center for AMS at Lawrence Livermore National Laboratory, which performs high-throughput, routine analyses of multiple nuclides, including ^{41}Ca *(4, 10)*. The automated AMS control system and streamlined sample preparation allow preparation and measurement of nuclides in more than 100 samples per day by a single operator.

Total AP, urea nitrogen, creatinine, potassium, sodium, chloride, carbon dioxide, phosphate, calcium, albumin, and glucose concentrations were measured with Beckman Synchron reagents on an LX20Pro analyzer. The PTH midmolecule was measured with a previously described assay *(27, 28)*. 25-Hydroxyvitamin D, 1,25-dihydroxyvitamin D, PTH (intact; detects amino acids 7–84), N-telopeptide, and bone-specific AP were measured by ARUP Laboratories (Salt Lake City, UT).

Analysis of ^{41}Ca in plasma by AMS was adapted from methods published by Lin et al. *(29)* and Freeman et al. *(30)*. Elimination of the microwave digestion step simplified the analysis. $^{41}Ca/Ca$ ratios were measured by AMS after calcium was isolated and purified using the steps described below.

We added 130 μL of 30 g/L (0.75 mol/L) calcium as $CaCO_3$ to 1 mL of plasma samples to ensure that there was sufficient calcium for AMS (~4 mg of Ca, or 0.1 mmol). Samples were then acid-digested for 30 min at room temperature with 2 mL of concentrated HNO_3. After digestion, 7 mL of deionized H_2O was added to the specimens; a yellow precipitant formed and was discarded after centrifugation (750*g* for 5 min), and the supernatant was retained. The calcium in the supernatant was then precipitated overnight with 1 mL of saturated ammonium oxalate and 3 mL of concentrated NH_4OH. Samples were centrifuged (750*g* for 10 min), the supernatant was discarded, and the resulting calcium oxalate was dissolved in 0.4 mL of 5 mol/L HNO_3. The samples were then diluted with deionized H_2O to an acid strength of 0.08 mol/L and chromatographed on a cation-exchange column as described below.

Cation-exchange columns (1.6 g of Bio-Rad AG 50W-X8, 200–400 mesh, hydrogen form) were prepared by washing with 5 mL of 5 mol/L HNO_3, 10 mL of deionized H_2O, and 5 mL of 0.08 mol/L HNO_3. The acid-digested plasma extracts were applied to the columns, which were then sequentially washed with two 4.5-mL portions of 0.08 mol/L HNO_3. The calcium was then eluted with two 4.25-mL portions of 5 mol/L HNO_3. The pH of the resulting solution was increased with 1.5 mL of deionized H_2O and 1.5 mL of concentrated ammonium hydroxide, and samples were allowed to cool for 30 min. After the samples were cool, 3 mL of concentrated HF was added, and CaF_2 was allowed to precipitate overnight. (Note: concentrated HF is extremely lethal, and all users must be properly trained before using this acid.) Samples were centrifuged, and the resulting CaF_2 was washed 3 times with 1 mL of deionized H_2O. After the H_2O was decanted, samples were placed in a 100 °C oven to dry for at least 12 h. Recovery of the added calcium was determined gravimetrically. Samples were then mixed with silver powder (2–4 parts CaF_2 to silver by weight) to improve conductivity in the AMS source and loaded into aluminum targets for AMS analysis.

As outlined in Fig. 1, AMS ^{41}Ca measurements were accomplished via (*a*) ionization of CaF_2/silver mixture, (*b*) selection of 97 or 98 *m/z* ions (target ions are $^{40}CaF_3^-$ and $^{41}CaF_3^-$, respectively), (*c*) acceleration of ions through 9×10^6 V, (*d*) removal of the 3 fluorine atoms and 9 electrons from the calcium atom through charge exchange in a thin carbon foil at the high-voltage terminal to destroy any molecular interferences, (*e*) a second stage of acceleration to ground potential, (*f*) measurement of $^{40}Ca^{8+}$ in an offset Faraday cup, and (*g*) ion identification and single-particle counting of $^{41}Ca^{8+}$ in a multianode gas ionization detector. The details of the AMS analysis can be found elsewhere *(4)*. Our analytical $^{41}Ca/Ca$ background value was $\sim 6 \times 10^{-14}$. Measurement precision was monitored by

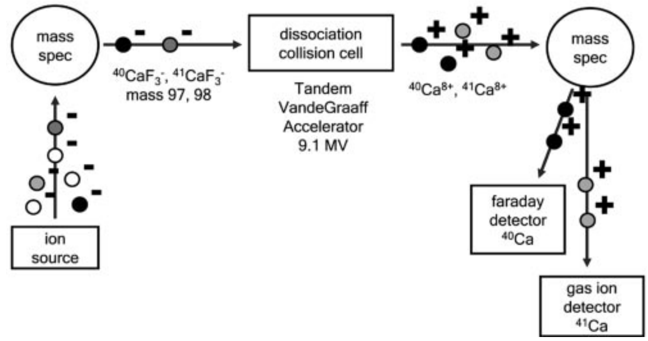

Fig. 1. Schematic of Lawrence Livermore accelerator mass spectrometer.

use of secondary standards similar in isotope ratio to unknowns and was <3%, assuming sufficient sample ^{41}Ca content to identify at least 1000 ^{41}Ca ions.

The method was validated at ^{41}Ca/Ca isotope ratios varying from blanks (~6×10^{-14}) to concentrations in the range expected 2 h post dose (9.08×10^{-10}). We added ^{41}Ca to these sera to achieve the target isotope ratios and processed them as described above. Within-run variability was determined at 6 isotope ratios (from blanks to 9.08×10^{-10}) with n = 3 at each concentration. Run-to-run variability was determined at 3 isotope ratios (from blanks to 9.08×10^{-10}) with each batch of samples. Run-to-run variability represented a minimum of 7 different batches over a 6-month period.

Results

Because the ^{41}Ca solution was given to human subjects, considerable effort was made to characterize it in terms of elemental composition, radioactivity, sterility, and pyrogenicity. The administered solution was free from heavy metal contaminants, and only 2 elements, sodium and calcium, were found at >1 part per million (Fig. 2); both had been added as part of the formulation process.

The radiologic purity of the ^{41}Ca master stock solution (1.5 μCi) was determined for the concentrated stock solution, rather than the diluted ^{41}Ca solution that was given to volunteers, to ensure maximized sensitivity and radiologic purity. In the master stock solution, neither γ nor β radiation above background was detected (Table 1). In fact, according to US Nuclear Regulatory Commission guidelines, the measured results of the prepared dosing solution fell below the limit of what is considered radioactive (2 nCi/g). As can be seen in Fig. 3, purity testing gave the expected low-energy internal bremsstrahlung emissions from the electron capture decay of ^{41}Ca and no measurable β or γ activity, thus demonstrating that the ^{41}Ca stock solution was radiologically pure. The maximum energy of the sample occurred at ~421 keV, a result consistent with the electron capture decay of ^{41}Ca to ^{41}K *(31)*.

Results of USP sterility tests showed no growth after 14 days, and USP endotoxin testing demonstrated <0.1 endotoxin units/mL. Taken together, these results demonstrate that the ^{41}Ca dose was radiologically and chemically pure and, importantly, also sterile and free of endotoxin.

The difference of results from expected values and the within-run imprecision of the method for ^{41}Ca/Ca ratios in serum samples with added ^{41}Ca over 4 orders of magnitude of isotope ratios expected when administering 10-nCi doses of ^{41}Ca to human volunteers are shown in Table 2. Run-to-run imprecision was in the 5% range with errors of 1%–3% (Table 3). As can also be seen in Table 3, with isotope ratios near background (~6×10^{-14}), it is difficult to routinely obtain good accuracy and precision because the background amount of ^{41}Ca is so small that it is difficult to detect, an ideal situation for labeling studies. However, after a single 10-nCi dose, volunteers had ^{41}Ca/Ca ratios several orders of magnitude greater than background; therefore, the poor precision and accuracy at background isotope ratios was not relevant to our studies. The mean recovery of calcium through the purification process was 87%.

There were no postdose changes in vital signs or pain ratings, compared with predose values, for any of the 8 volunteers. The mean baseline clinical chemistry values

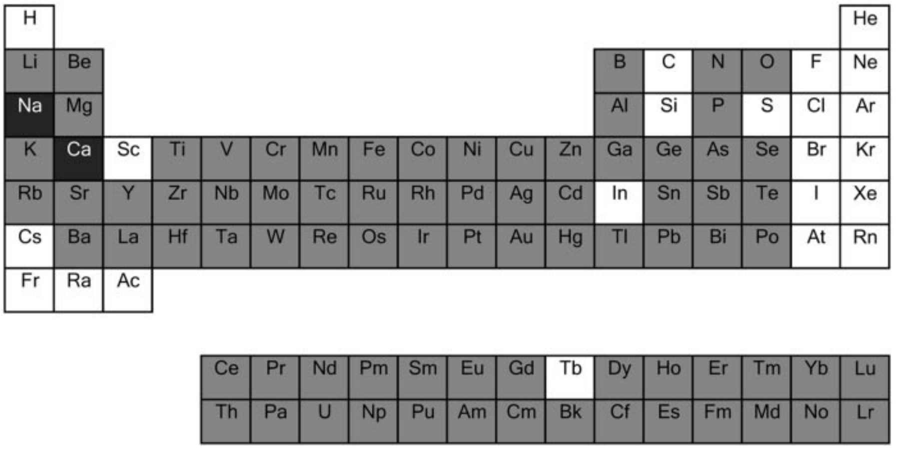

Fig. 2. Multielemental analysis of the administered ^{41}Ca solution.

White boxes indicate that elements were not analyzed; *gray boxes* represent elements that were present at <1 part per million; *black boxes* represent elements present at concentrations >1 part per million.

Table 1. Result of gamma/beta measurement of stock ^{41}Ca solution (1.5 μCi) showing no detectable counts above blank with 2 separate gamma/beta counters.

			Mean (SD) counts	
Instrument	Last calibrated	Calibration due	Blank	^{41}Ca stock
E120 A	January 2003	April 2004	45 (15)	45 (15)
E120 B	September 2002	December 2003	30 (10)	30 (10)

for the controls and the ESRD patients are shown in Table 4. The patients were hyperparathyroid, and the increased concentrations of N-telopeptide, bone-specific AP, and total AP provide evidence of increased bone turnover.

The initial distribution and elimination kinetics of the 10-nCi ^{41}Ca dose in the plasma from the controls (mean age, 54 years; n = 4) and the ESRD patients [mean age, 59 years; mean (SD) time on dialysis, 2.0 (1.1) years; n = 4] are shown in Fig. 4. As can be seen in Fig. 4, over the initial 28 days, the $^{41}Ca/Ca$ isotope ratio in plasma decreased more rapidly in the ESRD patients than in the controls, giving a lower area under the curve for ESRD patients compared with the controls ($P < 0.005$).

Discussion

Any time humans are exposed to radiation, the principle of ALARA (as low as reasonably achievable) applies. We administered 10 nCi (120 ng) of ^{41}Ca to 8 human subjects, 4 of whom were seriously ill. The biological risks associated with radiation exposure at this low exposure are too small to quantify. The only other published report of administration of intravenous ^{41}Ca to a human subject calculated that the overall radiation dose commitment was 0.06 μSv for the first year after a 10-nCi dose of ^{41}Ca and that this value is 30 000 times smaller than the total dose from natural radiation over this period *(32)*. In simple terms, the amount of radiation to which our volunteers were exposed is equivalent to that received from a 5-min commercial airplane ride *(33, 34)*. The amount of radioactivity that we administered was ~1000-fold less than that used in studies involving other calcium radioisotopes *(35)*.

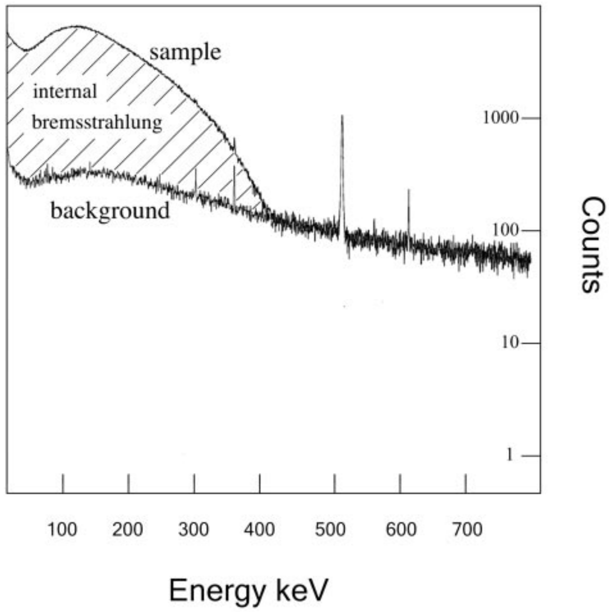

Fig. 3. Acquired energy spectrum for 2.06 g (18.0 μg of ^{41}Ca = 1.5 μCi) stock dose solution as measured for 1 week by use of a high-purity germanium detector.

No ionizing radiation (as α, β, or γ radiation) is produced; the low energy shown is from the electron capture decay of ^{41}Ca to ^{41}K.

Table 2. Within-run precision (n = 3) of $^{41}Ca/Ca$ measurements in serum samples.

Expected $^{41}Ca/Ca$	Measured $^{41}Ca/Ca$[a]	CV, %	Error, %
6.00×10^{-14}	$6.17 (0.30)\times10^{-14}$	5	3
4.55×10^{-13}	$5.01 (0.24)\times10^{-14}$	5	10
4.57×10^{-11}	$4.91 (0.17)\times10^{-11}$	3	7
9.14×10^{-11}	$9.61 (0.27)\times10^{-11}$	3	5
4.99×10^{-10}	$5.01 (0.04)\times10^{-10}$	1	<1
9.08×10^{-10}	$9.08 (0.05)\times10^{-10}$	1	<1

[a] Mean (SD); n = 3 at each concentration.

In addition to the perceived risk of radioactivity, other limitations of using $^{41}Ca/Ca$ ratios to monitor bone health include the high cost of instrumentation and the extensive sample preparation required for analysis. The transition of AMS from the research laboratory to a clinical laboratory may not occur in the near future, but it is worth remembering that the first mass spectrometers, with very limited capabilities, began as large complicated instruments relegated to sophisticated research laboratories. Today, many clinical laboratories have several mass spectrometers with a variety of ionization and analyzer configurations. At present, there is sufficient AMS beam time to meet current needs. If the methods we are developing become essential for monitoring bone health, additional resources will need to be developed. Alternative techniques for measuring ^{41}Ca include resonance ionization mass spectrometry *(36)* and atom trap trace analysis *(37)*, but these methods have not yet matched the throughput or sensitivity of AMS.

Before ^{41}Ca is used clinically, it must be demonstrated that measurement of the isotope ratio after a ^{41}Ca dose has clinical relevance. Our study is the first to show that $^{41}Ca/Ca$ ratios in a diseased group are different from

Table 3. Interassay imprecision of $^{41}Ca/Ca$ measurements.

Expected $^{41}Ca/Ca$	Measured $^{41}Ca/Ca$[a]	CV, %	Error, %	n
6.00×10^{-14}	$5.59 (2.78)\times10^{-14}$	50	7	22
4.57×10^{-11}	$4.70 (0.19)\times10^{-11}$	4	3	10
9.13×10^{-11}	$9.20 (0.39)\times10^{-11}$	4	1	18
9.08×10^{-10}	$8.81 (0.52)\times10^{-10}$	6	3	12

[a] Mean (SD).

Table 4. Mean (SD) baseline laboratory values for controls and ESRD patients.

Clinical test	Reference interval	Controls	ESRD patients	*P*
25-OH-vitamin D, ng/L	20–57	22 (8)	23 (15)	NS[a]
1,25-Vitamin D, ng/L	15–75	43 (18)	14 (5)	<0.05
PTH (intact), ng/L	15–75	40 (9)	800 (250)	<0.001
PTH (midmolecule), ng/L	5–70	13 (9)	1500 (750)	<0.01
N-Telopeptide, nmol BCE/L	5–24	11 (1)	210 (140)	<0.05
Bone AP, U/L	15–41	20 (3)	62 (17)	<0.01
Total AP, U/L	30–130	59 (13)	106 (32)	<0.05
Urea N, mg/L	80–180	160 (20)	630 (110)	<0.001
Creatinine, mg/L	4–12	9 (1)	106 (19)	<0.001
Potassium, mEq/L	3.5–5.0	4.4 (0.4)	5.5 (0.8)	<0.05
Sodium, mEq/L	135–145	140 (1)	137 (3)	NS
Chloride, mEq/L	95–106	106 (3)	100 (4)	NS
CO_2, mmol/L	24–31	27 (4)	23 (1)	NS
Phosphate, mg/L	25–45	36 (4)	53 (25)	NS
Calcium, mg/L	84–102	93 (2)	91 (4)	NS
Calcium mmol/L	2.1–2.55	2.32 (0.05)	2.27 (0.1)	NS
Albumin, g/L	32–46	41 (1)	35 (2)	<0.01
Glucose, mg/L	700–1100	950 (20)	1140 (200)	NS
Glucose, mmol/L	3.9–6.1	5.3 (0.1)	6.3 (0.1)	NS

[a] NS, not significant; BCE, bone collagen equivalents.

those in a control group. Other reports involving persons who received ^{41}Ca describe data from single individuals *(12, 13, 32)*. Using a 10-nCi ^{41}Ca dose, Johnson et al. *(32)* were able to measure ^{41}Ca/Ca ratios for more than 800 days and attributed small fluctuations in ^{41}Ca/Ca to menstrual cycle phases. Freeman et al. *(12)* compared calcium kinetics determined with dual stable isotopes with ^{41}Ca measurements and showed good agreement. They showed that a surgically menopausal female was losing ~100 mg of calcium from bone per day *(12)*. A separate publication by Freeman et al. *(13)* showed results suggesting that ^{41}Ca/Ca measurements had less variability than N-telopeptide and that ^{41}Ca/Ca ratios appear to decrease when bisphosphonates are administered *(13)*. Although these earlier studies were limited in size, they suggest intriguing possibilities for applications of this technology.

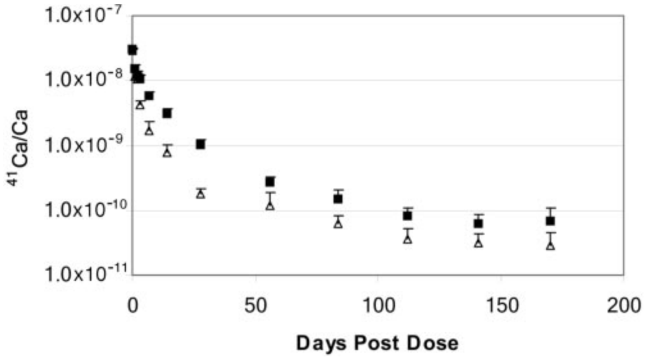

Fig. 4. ^{41}Ca is cleared more rapidly from blood by ESRD patients than by controls.

Kinetics of 10-nCi intravenous ^{41}Ca dose over 180 days in controls and ESRD patients. ■ and △ represent the mean (SD) values for ^{41}Ca/Ca in the controls (n = 4) and the ESRD patients on hemodialysis (n = 4), respectively. The area under the curve for the ESRD patients was significantly less than that for the controls ($P < 0.005$).

It is difficult to prove that the ^{41}Ca we administered ended up in the bone of the study participants, but several lines of evidence support this conclusion. It is well known that 99% of total body calcium is stored in bone. In addition, animal experiments have demonstrated that 24 h after administration of ^{45}Ca, an average of 85% of the absorbed calcium is found in the skeleton *(38)*. Using ^{41}Ca, we have shown that 4 weeks after mice received 0.55 nCi of ^{41}Ca, the concentration of ^{41}Ca was 5000 times higher in bone than other soft tissue *(39)*. Bronner et al. *(40)* showed that 5 days after adolescent boys received ^{45}Ca intravenously, the total quantity of ^{45}Ca excreted did not exceed 15% of the dose. These authors contended that nearly all of the calcium that enters the body at a given time is first retained, presumably in the skeleton *(40)*. It should be noted that initially after an intravenous dose of labeled calcium, most of the label is found in quiescent surface that is in dynamic equilibrium with blood *(41)*.

The rapid initial decrease in ^{41}Ca/Ca that we observed in plasma of ESRD patients indicates a faster disappearance of ^{41}Ca from the central compartment in the patients than in the controls. We hypothesize that the increased bone turnover in ESRD patients increases the available sites of mineralization and hence incorporation of the tracer into the bone formation process. This produces a more rapid initial clearance of ^{41}Ca from the serum compartment. One could also argue that the ^{41}Ca we administered to the ESRD patients was being cleared during hemodialysis, thus explaining the more rapid decrease of the tracer in these patients. Using ^{47}Ca, however, Cochran et al. *(35)* demonstrated that ESRD patients with hyperparathyroidism retained 94%–98% of the ^{47}Ca and that the dialysate and fecal radioactivity were minor components *(35)*. Clearly, more studies are needed with ^{41}Ca, but this technology offers the potential for monitoring calcium kinetics over a time period of years, using doses of tracer that pose a radiation risk that is too small to measure.

Management of renal osteodystrophy remains a difficult challenge faced by nephrologists. Current treatment guidelines suggest that in ESRD patients, nephrologists should aim to keep the circulating concentration of intact PTH in a range from 2 to 4 times the upper limit of the reference interval, although the form of PTH to be measured and the target concentration range remain controversial *(25, 42)*. The need for improved tools for managing renal patients is clear because the clinical methods currently used to measure renal osteodystrophy are indirect. The ability to directly monitor calcium metabolism may improve our understanding of bone health, which is increasingly recognized as a predictor of long-term outcomes in dialysis patients. In a recent review of the impact of mineral metabolism on mortality and morbidity in >40 000 hemodialysis patients, the attributable risk associated with disorders of mineral metabolism (18%) was greater than that for inefficient dialysis (5%) or anemia (11%) *(43)*.

In summary, we have characterized a dosing material in terms of chemical and radiologic purity in addition to sterility and pyrogenicity. This dose was administered to 8 human volunteers, and no adverse effects were observed. Using the methodology described in this report, we were able to measure $^{41}Ca/Ca$ isotope ratios for more than 170 days in volunteers who received 10 nCi of ^{41}Ca. The data presented show that this method can be used to monitor differences in calcium kinetics between healthy individuals and patients with ESRD and hyperparathyroidism.

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